

Art 34

C L A I M S :

(amended)

Emb 1

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1. A method for the withdrawal of one or a few components of a system, such as molecules, molecular complexes, vesicles, micelles and/or cells, together with an associated withdrawal volume element, V , with $10^{-9} \text{ l} \geq V \geq 10^{-18} \text{ l}$, from a sample volume containing the components to be withdrawn by transferring the component or components to another environment wherein space and time of the withdrawal are determined by a signal correlating with the component to be withdrawn, which signal is established by an optical analytical system, in particular based on confocal laser correlation spectroscopy or near field spectroscopy, which is capable of analyzing specific molecular properties in volume elements as small as 10^{-14} l .

2. The method according to claim 1, characterized in that said sample volume is connected with said other environment in the form of a receptor means through a pore of a capillary or membrane wall whose smallest aperture D is defined by $100 \mu\text{m} \geq D \geq 0.1 \mu\text{m}$.

a 3. The method according to ^{claim 1} ~~claims 1 and/or 2~~, characterized in that at least one withdrawal to the same receptor means is performed in one or more steps wherein the individual withdrawal processes are performed independently in terms of a gathering process.

a 4. The method according to ^{claim 1} ~~at least one of claims 1 to 3~~, characterized in that said withdrawal is effected through a directed transport of said volume element by means of at least one electrical voltage or field strength impulse and/or mechanically by means of at least one pressure difference impulse and/or by means of at least one light ~~pressure impulse~~.

to
be
used

a 5. The method according to ^{claim 2} ~~at least one of claims 2 to 4~~, characterized in that said receptor means is a capillary the lumen of which is larger than the diameter of the pore or capillary tip the aperture of which is in direct contact with the sample volume.

a 6. The method according to ^{claim 1} ~~at least one of claims 1 to 5~~, characterized in that said withdrawal is performed selectively by means of an electrical field strength impulse by shortly applying an electrical field at least once for electrophoresis of electrically charged components and/or for electroosmosis with coupled transport of electrically neutral molecules wherein one electrode is in electrically conducting contact with the solution on the side of the sample volume while the ^{other} ~~other~~ electrode is in electrically conducting contact with the solution on the side of the receptor means and the conducting contact between the two compartments is established through the pore.

a 7. The method according to ^{claim 1} ~~at least one of claims 1 to 6~~, characterized in that said withdrawal is performed selectively by means of a mechanical pressure pulse by at least one short increase of the pressure in the volume outside the receptor compartment as compared to the pressure inside the receptor compartment, and/or by a short reduction of the pressure inside the receptor compartment.

8. The method according to claim 7, characterized in that the reduced pressure pulse is caused by a piezo-controlled dispenser module, the filling volume of which is inside the receptor compartment, and/or the pressure pulse and/or reduced pressure pulse is caused by the volume of the receptor means being enlarged by a change of the piston position of a coupled piston pump device, preferably controlled by a stepping motor, or the sample volume is diminished in favor of the receptor means.

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for many a minutes

- claim 6*
a 9. The method according to ^{claim 6} ~~at least one of claims 6 to 8~~, characterized in that the size of the received volume is controlled by the number of the dispensed droplets or the steps of the stepping motor.
- claim 1*
a 10. The method according to ^{claim 1} ~~at least one of claims 1 to 9~~, characterized in that said correlating signal triggers the withdrawal time which is in that moment when the particle or particles to be withdrawn is/are present within the withdrawal volume with high probability.
- claim 1*
a 11. The method according to ^{claim 1} ~~at least one of claims 1 to 10~~, characterized in that said measuring volume is a subvolume of said sample volume.
- claim 2*
a 12. The method according to ^{claim 2} ~~at least one of claims 2 to 11~~, characterized in that the time and/or space specific correlation between the analysis of said measuring volume within volume element V and the withdrawal of a desired component by removing the volume element V is effected with the aid of a computer/software means wherein at least one component which has been analyzed in volume element V will be present in the withdrawn volume element V during the withdrawal process wherein the pore of the receptor means is mechanically approached to the volume element, and/or the volume element V or parts thereof are transported to the pore of the receptor compartment with a predetermined time correlation by transport in the flow or by electrostatic or magnetic field gradients, and/or the analysis is performed immediately in front of the pore of the receptor compartment.
13. The method according to claim 1, characterized in that said measuring volume is smaller than the volume element V.
- claim 1*
a 14. The method according to ^{claim 1} ~~at least one of claims 1 to 13~~, characterized in that a number of coupled analytical and

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withdrawal processes are sequentially employed in a cascade-like manner wherein the withdrawn sample volumes are subsequently again subjected to an analysis with or without an intermediate dilution step, and can be withdrawn again after completion of the analysis in enriched form by a second and/or further withdrawal unit.

a 15. The method according to ^{Claim 1} ~~at least one of claims 1 to 14~~, characterized in that those components are withdrawn which will form spectroscopically detectable complexes with at least one reagent.

a 16. The method according to ^{Claim 1} ~~at least one of claims 1 to 15~~, characterized in that components are withdrawn which have been unknown with respect to their molecular nature, such as molecules, cells, vesicles, molecular complexes, which can be identified through an interaction with known structures, or through activities, such as enzymatic activity, or complex formation.

17. The method according to claim 16, characterized in that the unknown particles are pathogens or immunogens which are selectively withdrawn by collecting serums from at least one organism wherein at least one serum (serum 1) is obtained from the phase of an acute infection by a pathogen or immunogen which is optionally as yet unidentified (unknown), and at least one serum (serum 2) is obtained from the same or at least one other organism with the same or a homologous infection in the phase of chronic disease wherein said optionally unknown pathogen or immunogen from serum 1 is induced to measurable complex formation with indirectly or immediately fluorescence-dye labeled antibodies from serum 2.

a 18. The method according to ^{Claim 16} ~~at least one of claims 16 to 17~~, characterized in that the simultaneous binding of ligands giving different fluorescence signals, e.g. labeled anti-

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bodies from different organisms, is determined by cross-correlation.

a 19. The method according to ^{Claim 16} ~~at least one of claims 16 to 18~~, characterized in that the labeling of said antibodies is done immediately by at least one reaction with dyes capable of coupling, or indirectly by reaction with dye-labeled antibody binding domains, in particular protein A derivatives or protein G derivatives.

a 20. The method according to ^{Claim 1} ~~at least one of claims 1 to 19~~, characterized in that said optionally unknown particles are per se known microorganisms or vesicles wherein the detected characteristics are specific interactions with surface-expressed or cytosolic-expressed structural elements of natural or recombinant proteins or peptides or enzymatic activities with fluorescence-labeled target molecules.

a 21. The method according to ^{Claim 1} ~~at least one of claims 1 to 20~~, characterized in that said measuring volume is composed of measuring subvolumes illuminated in parallel wherein the simultaneous illumination of a multitude of measuring volumes is effected by at least one electromagnetic radiation source using at least one holographic grid.

22. The method according to claim 21, characterized in that the registration of fluorescence signals from at least one measuring volume is performed by confocal focussing using a multitude of confocal pinhole apertures in the object plane, or by coupling the signals into optical waveguides in the object plane, or by multiarray detectors in the object plane.

a 23. The method according to ^{Claim 21} ~~at least one of claims 21 to 22~~, characterized in that for performing parallelized measurements on at least two measuring volumes, at least two

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measuring volumes in common or assembled in groups are focussed confocally onto at least one detector element of a photon-registrating measuring element in the object plane in the signal registration.

- a 24. The method according to ^{claim 1} ~~at least one of claims 1 to 23~~, characterized in that for detecting very low concentrations of fluorescing molecules, the sample volume is subjected to a scanning process prior to the actual measuring and withdrawal of at least one component wherein the time required for detecting a sought particle is shortened by varying the space coordinates of the measuring volume with respect to the space coordinates of the sample volume continuously or discontinuously in time.

- a 25. The method according to ^{claim 1} ~~at least one of claims 1 to 24~~, characterized in that the time interval δt for the measurement of one or more volume elements having defined space coordinates prior to the detection of a sought molecule by its fluorescence measuring signal is shorter than the average dwelling time of the sought molecule within a measuring volume.

- a 26. A device comprising a receptor means having a pore and a measuring device for the illumination and/or measurement of a measuring volume by electromagnetic radiation for performing the method according to ^{claim 2} ~~at least one of claims 2 to 25~~, characterized in that said pore of the receptor means can be approached closely to the measuring volume which is smaller than 10^{-14} l and said receptor means is connected with a mechanically, optically or electrically controllable withdrawal device.

- a 27. The device according to claim 26, consisting of an arrangement of a closed or open container for receiving a sample volume and at least one connection to at least one second volume element which is in direct contact with the sample

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volume through an aperture and a liquid phase wherein said aperture is preferably immediately adjacent to said measuring volume.

claim 1

a 28. Use of the method according to ~~at least one of claims 1 to~~
a ~~27~~ for the preparative recovery of unidentified pathogens,
immunogens or organisms which functionally express parts of
a genome.

claim 1

a 29. Use of the method according to ~~at least one of claims 1 to~~
a ~~25~~ for the preparation of genetic probes for the identifi-
cation/detection/cloning of functional elements of a whole
genome and/or diagnostic and/or therapeutic agents derived
therefrom.

claim 1

a 30. Use of the method according to ~~at least one of claims 1 to~~
a ~~25~~ for the analysis and preparative recovery of nucleated
fetal cells from maternal blood.

claim 1

a 31. Use of the method according to ~~at least one of claims 1 to~~
~~25~~ for the detection and preparative recovery of at least
one specific gene of a microorganism the corresponding at
least one gene product of which is presented on the inner
or outer cell membrane or viral envelope.

claim 1

a 32. Use of the method according to ~~at least one of claims 1 to~~
a ~~25~~ for the determination of the function of gene products
of defined gene segments.

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